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Some Physical and Chemical Properties of Humic Substances as a Basis of Their Characterization

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Abstract: One way to demonstrate the participation of phenols derived from lignin degradation and from microbial synthesis, on the one hand, together with nitrogenous protein degradation products, on the other, in formation of humic substances is the oxidative or reductive cleavage, whereby different types of phenols can be identified. The difficulties in isolating humic acids are caused by the mixture of various organic compounds in soils with similar properties. The solvents have a decisive influence on the results of isolation and fractionation.

The function of oxygen in the humic fractions concerns mainly acidic groups.

The humic acids can be fractionated by flocculation with neutral salts in "gray" and "brown" humic acids which differ in their elemental composition and in some physical properties. By infrared spectroscopy it was shown that a smectic mineral is the main constituent of the ash of the "gray" humic acids fraction.

The differences of the distribution of nitrogen in different fractions of humic acids is discussed in connection with light absorption. The nonhydrolyzable part of nitrogen seems to be most effective. An example for separation of humic substances by gelfiltration is given.

The dependence of some molecular properties such as aggregation and dispersion of humic fractions from the conditions present was demonstrated with some chemical and physical methods, e.g., viscosimetry and ultracentrifuge.

The humification of material from dead organisms is the first step in the formation of carbonaceous material with a higher carbon content than the living organisms. Therefore, these processes are also important for organic geochemistry.

1. Structure of humic substances

In Fig. 1, the present knowledge about the formation of humic substances from transformed constituents is depicted.

Plants and microorganisms are the most important organisms for humification processes. The most important constituents of the organisms are carbohydrates, proteins, phenolic compounds such as lignin and others which are transformed to the monomers and their degradation products. They were used as carbon and nitrogen sources for the microorganisms or formation of humic substances.

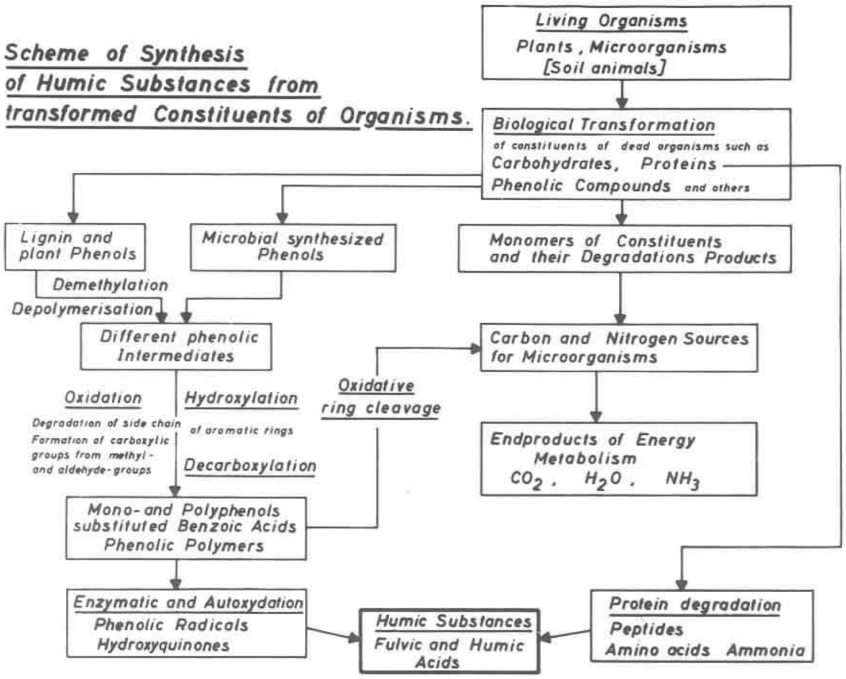
Scheme of Synthesis of Humic Substances from transformed Constituents of Organisms.
Living Organisms
Plants, Microorganisms
[Soil animals]
Biological Transformation
of constituents of dead organisms such as
Carbohydrates, Proteins
Phenolic Compounds and others
Lignin and plant Phenols
Microbial synthesized Phenols
Monomers of Constituents and their Degradations Products
Demethylation
Depolymerisation
Different phenolic Intermediates
Carbon and Nitrogen Sources for Microorganisms
Oxidation
Hydroxylation
Oxidative ring cleavage
Degradation of side chain
Formation of carboxylic groups from methyl- and aldehyde-groups
of aromatic rings
Decarboxylation
Endproducts of Energy Metabolism
CO2, H2O, NH3
Mono- and Polyphenols
substituted Benzoic Acids
Phenolic Polymers
Enzymatic and Autoxydation
Phenolic Radicals
Hydroxyquinones
Humic Substances
Fulvic and Humic Acids
Protein degradation
Peptides
Amino acids Ammonia

Fig. 1. Scheme of synthesis of humic substances from transformed constituents of organisms

Carbon dioxide, water, and ammonia are the end products of the energy metabolisms. Phenolic compounds can be derived from lignin and other plant phenols or can be synthesized by microorganisms. Lignin is demethylated and depolymerised. Thereby different phenolic intermediates are formed.

A proof of this concept is the degradation of humic substances and the identification of the formed compounds (Fig. 2). The main reactions are:

oxidation,
hydroxylation,
decarboxylation.

In this way mono- and polyphenols, substituted benzoic acids, and phenolic polymers are formed.

Aliphatic compounds are formed by oxidative ring cleavage. These can be used by the microorganisms as a carbon source, and they disappear in this way from the mixture of compounds which form humic substances by different processes.

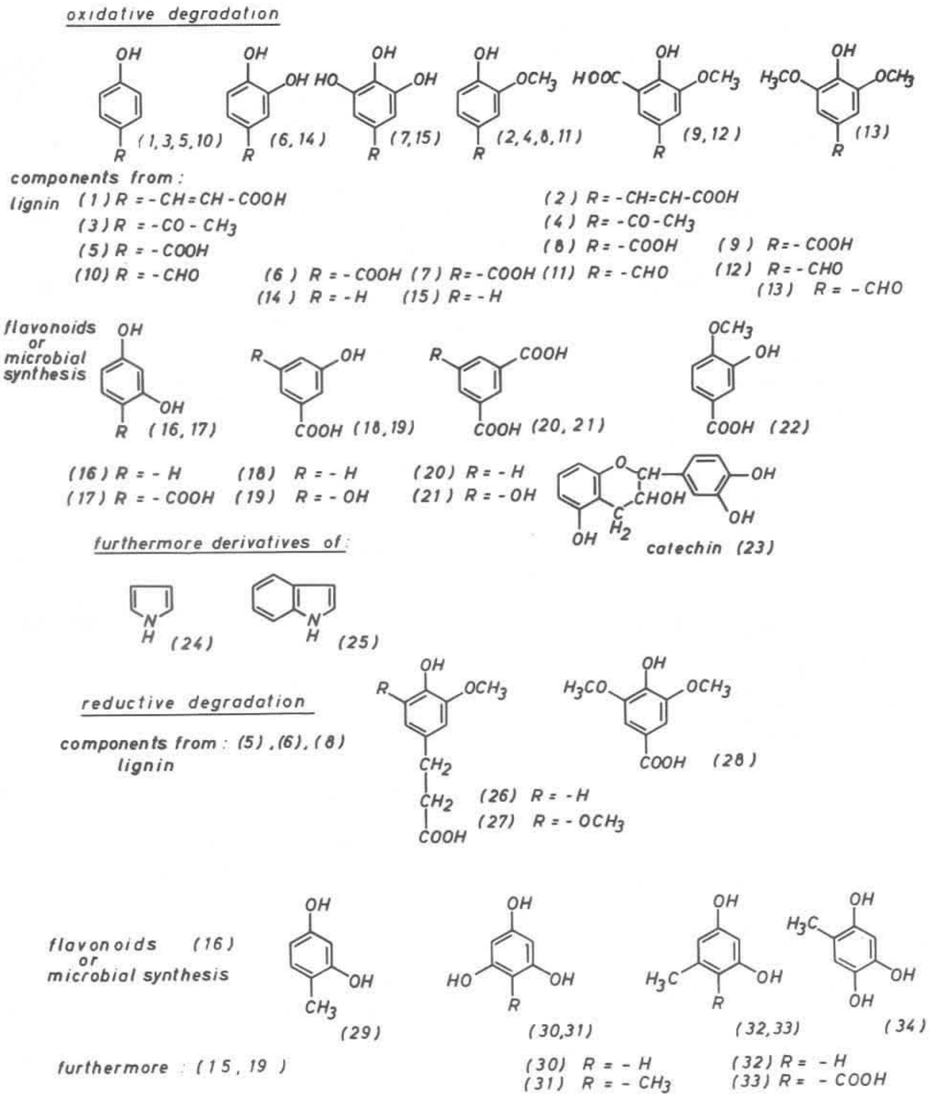

Fig. 2. Degradatation products of humic acids

I. = 2 % HCl cold, 1 % NaOH, cold: Nehring (1955), Tyurin (1937)
II. = 5 % HCl, 70 °C, 0,5 % NaOH, boiling: Springer (1938)
III. = 5 % HCl, 70 °C, 1 % NaOH, cold
IV. = 0,1 M $Na_4P_2O_7$, 0,1 N NaOH, 16 hours, cold: Kononova and Belchikova (1961)
V. = 0,1 N $Na_4P_2O_7$, 0,1 N NaOH, 16 hours, cold
VI. = 0,1 N $Na_4P_2O_7$, 8 hours, boiling: Welte (1956)
VII. = 0,1 N $Na_4P_2O_7$, 5 % hydryzinehydrate, 16 hours, cold

Enzymatic and autoxidation lead to radicals and hydroxyquinones. Condensation reactions occur between the mentioned phenolic compounds and protein degradation products such as peptides, amino acids, and ammonia. In this way humic substances are formed which are mainly divided into fulvic, humic acids, and humins by different separation operations.

To determine phenolic structure units of humic acids two methods mainly are used.

1. Oxidation of humic acids in alkaline solution with nitrobenzene at 120–140 °C. Yield up to 7 % (Morrison, 1963).
2. Reductive cleavage with sodium amalgam in oxygen free medium at 100–120 °C (Burges, Hurst, and Walkden, 1963) yield up to 30 % of mixtures of aldehydes. This is the prefered method.

By oxidative as well as by reductive cleavage, mainly two types of degradation products are found.

1. Phenols, which can be derived from lignin.
2. Phenols, which can be derived from flavonoids or are synthesized by microorganisms.

In the first case, the phenolic hydroxyl groups are in o-position, in the second case mostly in m-position. The phenolic polycarboxylic acids can be derived from phenolic units which are bound by side chains or by rings. The structure of the degradation products demonstrates that the humic acids consist of a larger number of phenolic units which are connected in different manners. Also nitrogenous compounds have been found such as pyrrol- and indolderivatives; this means that in humic acids nitrogen is also partly bound in heterocyclic form.

In recent times polycyclic aromatic compounds were indentified by zinc dust distillation of humic acids. The numbers given by Hansen and Schnitzer (1969) are naphthalene (0,1 %), phenanthrene (0,1 %), anthracene (0,2 %), pyrene (0,1 %), perylene (0,2 %), and benzanthrene. Different other authors obtained similar results (Blumer 1961, Kumada and Suzuki, 1961; Cheshire et al., 1967). These phenolic aromatic compounds may also partly be formed by the high temperature over 200 °C during zinc dust distillation.

2. Problems of isolation of humic substances

The substances formed by humification of plant material consist of a mixture of various compounds with similar properties. Therefore, the extraction of substances with a uniform composition is very difficult. The separation procedures lead to different results dependent upon the physicochemical properties of the extracted materials as well as upon the extractants.

Many extractants were used for isolation of humic substances (Scheffer and Ulrich, 1960; Tinsley and Salam, 1961; Dubach and Mehta, 1963). The solvents can be derived according to their chemical and physical efficiency in several groups: (1) (1) acids, (2) strong alkali, (3) complexing agents, and (4) organic solvents.

The properties of the solvent have a decisive influence on the result of fractionation. In Fig. 3, different procedures of extraction are summarized (Wiesemüller, 1965).

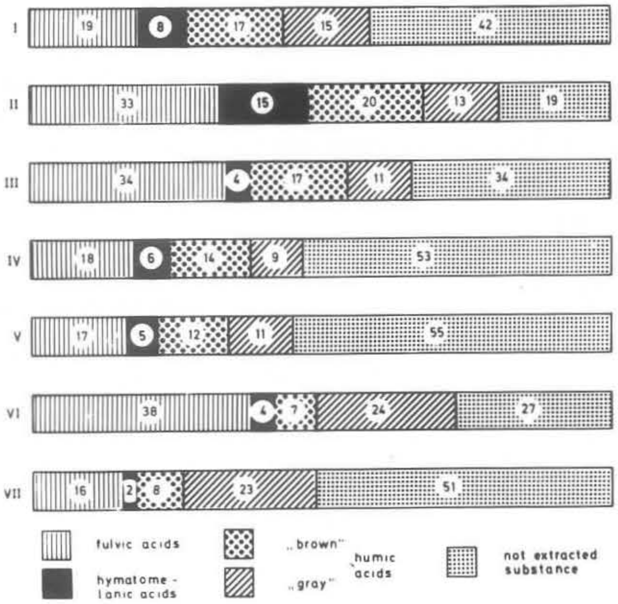

Fig. 3. Comparison of different extractants (data in % of total carbon of chernozem extract) (Wiesemüller 1965)

Several factors are remarkable:

1. Different quantities of humic substances are extracted by the different solvents. This is due of the quantity and type of inorganic parts of the soil.
2. The ratio of fulvic acids to the sum of "brown" and "gray" humic acids varies according to the procedure of extraction.
3. The ratio of "brown" to "gray" humic acids depends on the method of extraction.

These facts should lead to the conclusion that all authors should use a standardized, conventional method and that it is possible to compare the results of different investigations. (Flaig, 1964; Kononova, 1961). To organize such a project would be the task of the organic geochemistry group. The elemental composition depends on environmental conditions such as the plant cover, the climatic conditions, the inorganic soil constituents, soil management of man and others. Such differences will be found principally in all systems from which humic substances are isolated (Kononova, 1961).

3. Function of oxygen

As an example for elemental analysis, the data of humic and fulvic acids from different horizons of a podzol are depicted in the bellowing table.

From Table 1, it is easy to see that the carbon content of humic acids is higher and the oxygen content is lower in the case of fulvic acids. This is in accordance with the total acidity and may be mainly caused by the higher carboxyl content of fulvic acids. Similar differences exist also in the case of carbonyl groups.

The nitrogen content of fulvic acids is generally lower than that of humic acids. The aromaticity of the total organic fraction of a podzol A_0- and B_h-horizon was estimated according to the method of Mazumdar et al. (1957) and Chakrabartty et al. (1960), and Wright and Schnitzer (1961) (Table 2). The preparations were exposed to an airstrem of 170 °C during 500 hours. The aromatic structures are more resistant to oxydation than the aliphatic and alicyclic.

Table 1. Analytical Characteristics of Humic and Fulvic Acids from a Podzol B-horizon (Schnitzer, 1965; Schnitzer and Gupta, 1964)

	Elementary composition	
Element	Humic acid %	Fulvic acid %
C	56.72	50.92
H	5.21	3.34
N	2.37	0.74
S	0.35	0.26
O (by difference)	35.35	44.74
Oxygen containing functional groups (m-equivalent per g dry ash-free material)		
Total acidity	5.7	12.4
Carboxyl	1.5	9.1
Total hydroxyl	6.9	6.9
Phenolic hydroxyl	4.2	3.3
Alcoholic hydroxyl	2.7	3.6
Carbonyl	0.9	3.1
Molecular weight*)	1684	669

*) The average molecular weights were determined by Schnitzer and Des Jardins (1962) by lowering of freezing point in sulfolane.

Table 2. Distribution of the Carbon and Hydrogen Atoms in the Organic Frations of Two Podzol Horizons (Wright and Schnitzer, 1961)

	Carbon in %			Hydrogen in %		
Horizon	aromatic	in COOH-groups alicylic	aliphatic and/or	aromatic	in COOH-groups	aliphatic and/or alicylic
A_0	49	3	48	36	3	61
B_h	48	22	30	21	28	51

As the amount of aliphatic and/or alicyclic carbon and hydrogen is higher in A_0-horizon than in B_h-horizon, the organic fraction in the B_h-horizon has a more aromatic structure than the A_0-horizon. The carboxyl content is higher in the B_h-horizon.

Similar results are obtained by alkaline oxidation with sodium permanganate or with nitric acid of humic acids and determination of the isolated aromatic compounds (e.g., Schnitzer and Wright, 1960).

The acidic groups of humic acids (Table 3) participate in exchange capacity, which is lower in the case of podzol than in the case of chernozem. The exchange capacity of fulvic acids is lower than that of humic acids and is dependent upon the pH-value and the type of cations. Further investigations have been made to differentiate the fraction of humic acids.

Table 3. Exchange Capacity (meq/100 g) of Humic Acids in Dependence of pH (Rydalewskaja and Tischenko, 1941)

Soils from which humic	pH = 4,5		pH = 6,4		pH = 8,1	
acids have been isolated	meq	rel	meq	rel	meq	rel
Chernozem	292	(100)	433	(149)	590	(203)
Podzol	243	(100)	410	(169)	549	(226)
Peat Soil	170	(100)	286	(168)	400	(235)

4. Fractionated precipitation of humic acids with neutral salts ("gray" and "brown" humic acids fractions)

By flocculation with sodium chloride at pH-values between 7 and 8, purified humic acids can be separated in two fractions. The precipitated fraction is called "gray" humic acids, the part which remains in the solution and is precipitated with hydrochloric acid is the "brown" humic acids fraction (see Flaig et al., 1955). By electrophoresis similar fractions are obtained.

The quantitiy of "gray" and "brown" humic acids fractions (Table 4) is different according to soil type from which the humic acids have been isolated. Both fractions differ remarkably in their properties.

Table 4. Separation of Humic Acids in "Gray" and "Brown" Humic Acids Fraction by Precipation with Sodium Chloride in Percent of Total Humic Acids. (Scharpenseel and Krause, 1962)

Chernozem, Hungary		Podzol, Germany B_h-horizon	
"Gray"	"Brown"	"Gray"	"Brown"
humic acids fraction		humic acids fraction	
68,5 %	31,5 %	28,1 %	71,9 %

Table 5. Differences in the Chemical Composition of "Gray" and "Brown" Humic Acids Fractions (values of ash-free substances, oxygen separately determined) (Flaig, Scheffer, and Klamroth, 1955)

Chernozem	C %	H %	O %	N %	Sum	OCH_3	Ash
Total-h.a.	57,78	3,25	34,32	3,90	99,52	0,95	9,60 brown-red
G-Fraction	61,60	2,79	32,42	2,83	99,64	0,78	10,18 dark-brown
B-Fraction	56,97	4,25	33,66	4,19	99,07	1,60	3,42 carmine-red
Podzol							
G-Fraction	61,62	2,96	33,93	2,18	100,89	0,00	5,26 carimine-red
B-Fraction	59,40	3,07	36,34	1,89	101,10	0,27	2,38 gray

In the "gray" humic acids fractions, (Table 5), the carbon and the ash content are higher and the oxygen, the nitrogen, and the methoxyl content is lower than in the "brown" humic acids fraction.

In connection with the separation of lignin-like products from humic acids with organic solvents, it might be mentioned that a methoxyl content of 1,5 % would correspond a lignin content of 10 % with a methoxyl content of lignin of 15 %.

5. Characterization of the ash in the G-fraction

Normally the content of the "gray" humic acids fractions is higher than that of the "brown" humic acids fractions. Also the comparison of the ashes of the two types of humic acids is different. The ash of the preparations of the total humic acids has the following composition: 70 % SiO_2, 20 % Al_2O_3, and 10 % Fe_2O_3.

With infrared spectroscopy, the ash was characterized. The investigation shows also the possibilities of the usage of infrared spectroscopy for characterization of fractions of humic acids (Rietz unpublished). The results with this method are limited.

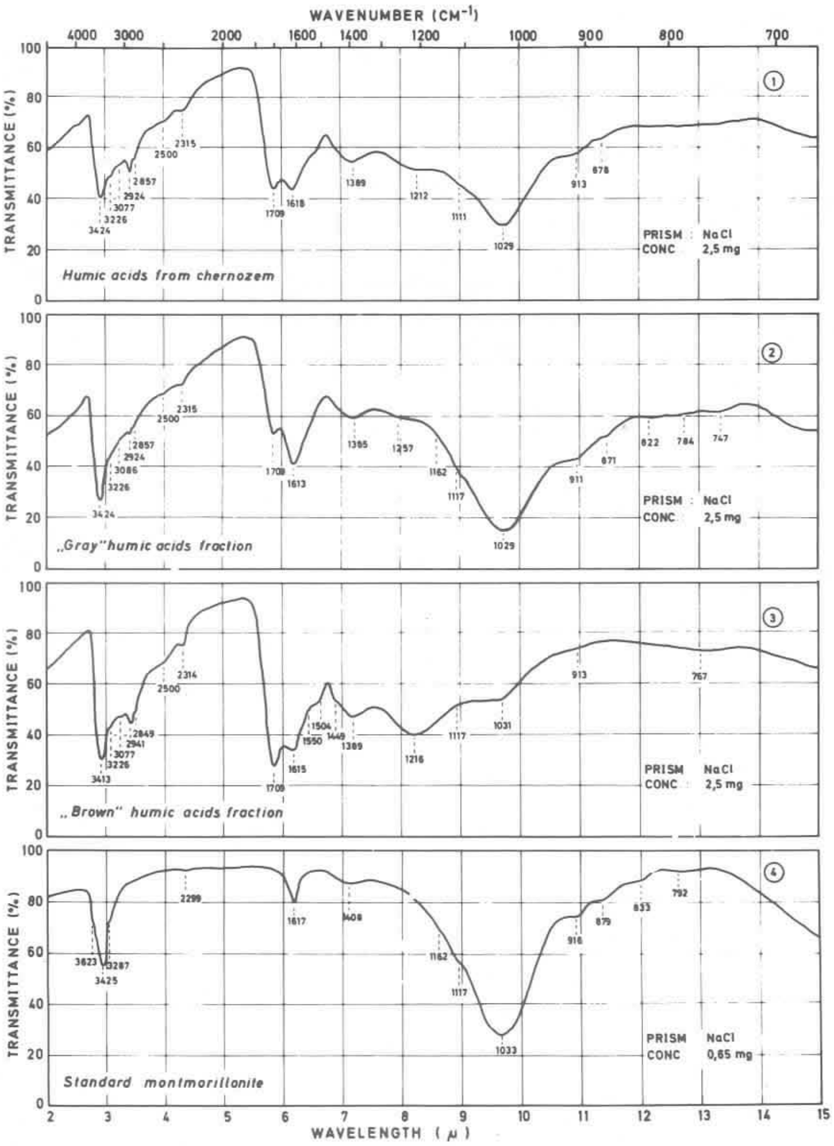

Fig. 4. Infrared spectr:. of fractions of humic acids and of montmorillonits.

1. "Total"-humic acids
2. "Gray"-humic acids fraction
3. "Brown"-humic acids fraction
4. "Standard"-montmorillonite

Without going in to details, the comparison of the spectra (Fig. 4) shows that a strong absorption occurs in the case of the "total" (1) and of the "gray" humic acids fraction (2) in the range of the Si-O-Si vibrations at 9.72 μ (1029 cm^{-1}). These investigations and also those with the electron microscope give evidence to the presence of a smectic mineral of the type of montmorillonite (4).

The fractionation results also in a decrease of the C = O-vibrations at 5,85 μ (1709^{-1}) in the case of the "gray" humic acids fraction (2) and in an increase in this of the "brown" humic acids fraction (3). The maximum at 7.20 μ (1389 cm^{-1}), and the absorption of carboxylate groups indicates a higher content of carboxyl groups in the "brown" humic acids fraction (3) than in the "gray" humic acids fraction (2). This could also be concluded by the above mentioned results.

6. Distribution of nitrogen

The nitrogen content of humic acids is between 1 and 5 %. By hydrolysis with mostly 6 N HCl (Bremner, 1965) the following can be split off:
20–40 % as α-NH_2-N, 10–25 % as NH_3-N and 1–5 % as amino sugar-N. At least 7–12 % of the α-NH_2-N is in peptide linkage.

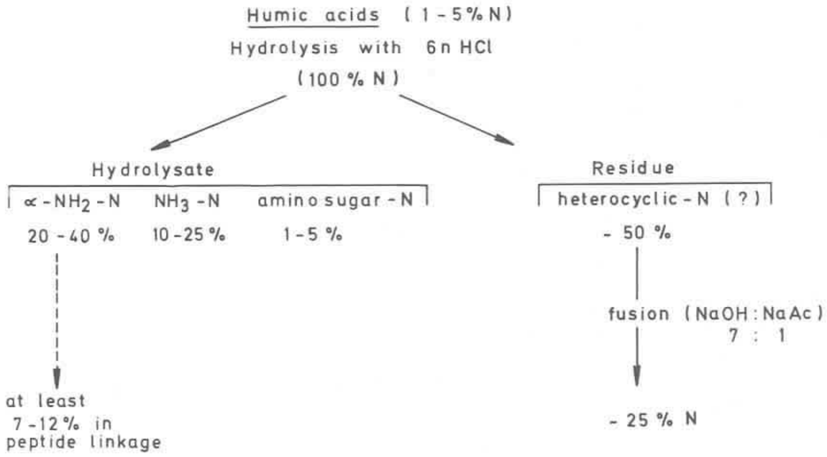

Fig. 5. Distribution of nitrogen in humic acids

In the residue of hydrolysis, about 50 % N remain which are supposed to be bound to a large extent in heterocyclic form. In a fusion with a mixture of sodium hydroxide and sodium acetate up to 2,5 % N can split off again. According to model experiments with phenylglycine, a part belongs to amino acids which are not hydrolized by 6 N HCl (Flaig and Breyhan, 1956). These data are mentioned because they are needed for the discussion of some properties of fractions of humic acids such as light absorption.

The humic acids from chernozem contain mainly more nitrogen than those from podzol. The content of α-amino-nitrogen is lower in chernozems than in podzols (Bremner, 1955a).

The part of nitrogen, which can be split off by hydrolysis (Table 6) is smaller in the case of "gray" humic acids. The hydrolyzable part of the "brown" humic acids consists mainly of α-amino-nitrogen. According to these results, there must be a difference of composition between "gray" and "brown" humic acids. In connection with the following considerations, it is mentioned that nearly no difference exists in the nitrogen content of the residues of hydrolysis of both types of acids.

Table 6. Function of Nitrogen in "Gray" and "Brown" Humic Acids Fractions (Bremner, 1955b)

	G-fraction	B-fraction
% total-N (ash free)	2,29	4,41
Distribution of N *after hydrolysis* (total-N = 100)		
hydrolyzable	23,6	69,1
NH_3-N	7,7	9,8
α-NH_2-N	10,5	37,5
not hydrolyzable	76,4	30,9
Residue of hydrolysis		
% of initial material	79,6	56,3
% N (ash free)	2,05	2,10
% ash	7,7	0,2
% N of total-N of initial material	58,2	30,0

7. Differences in light absorption of humic fractions

The considerations about the participation of the differently bound nitrogen in light absorption will be explained with the schematic presentation of the electronic spectra of fractions of humic acids (Fig. 6).

The proteins do not absorb in the visible range and therefore cannot give a contribution to the colour of the humic acids. The light absorption in the visible range cannot be dependent upon the content of total nitrogen by this reason.

Generally the humic acids from chernozem rich in nitrogen have not only a higher light absorption but also a stronger at longer wavelengths. The stronger light absorption of humic acids from chernozem may be caused by a larger part of the nonhydrolyzable nitrogen. It can be assumed that these humic acids have a larger

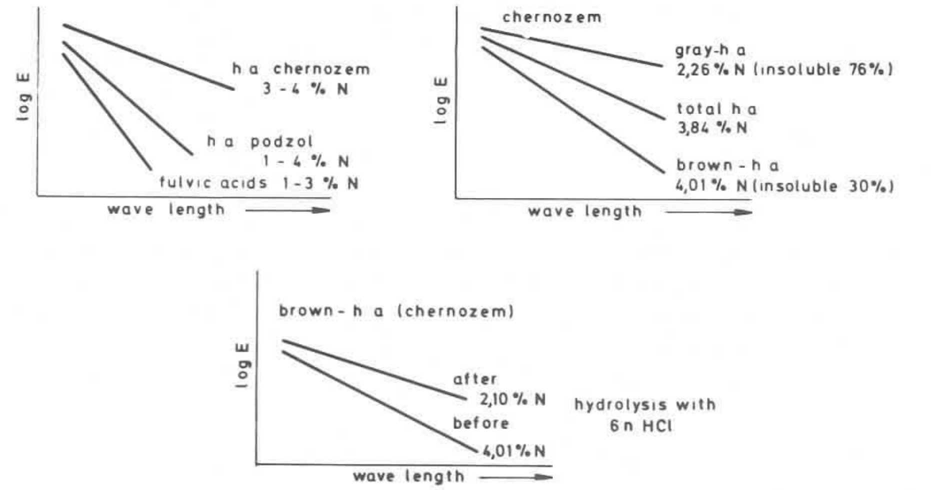

Fig. 6. Scheme of absorption spectra of humic acids isolated from chernozem or podzol and of their fractions as well as of fulvic acids (Flaig 1967)

content of "heterocyclic" parts of the molecule with a bathochromic effect than those from podzol. This assumption may be confirmed by the fact that the fraction of "gray" humic acids with its lower total nitrogen content of 2.25 % but with its larger content of not hydrolyzable nitrogen has a stronger light absorption than the "brown" humic acids fraction with the higher total nitrogen content of 4,01 % and its weaker light absorption.

Furthermore, also the residue of hydrolysis of the "brown" humic acids fraction with a nitrogen content of 2,10 % has a stronger light absorption than the non-hydrolyzable fraction with 4,01 % nitrogen.

The nearly monotonously increasing absorption in the visible and ultraviolet range cannot be explained only by the presence of "heterocyclic" parts of the molecule. So Kleist and Mücke (1966) suppose that the higher extinction of the "gray" humic acids fraction is caused by its larger content of radicals. The result that humic acids contain radicals (Steelink and Tollin, 1962; Steelink, 1964; Kleist, 1967) led to the assumption that they are systems of intramolecular quinhydrones which are stabilized by mesomery.

The quotients of extinction in small ranges of wavelengths are used for the characterization of humic fractions or systems, whereby similarity degrees are determined with statistical method (Salfeld, 1965, 1968, 1971; Söchtig and Salfeld, 1971). Investigations about characterization of humic substances have also been made by other authors (Kumada, 1965; Orlov and Grindel, 1967).

8. Gelfiltration

It is not possible to discuss here the advantages and disadvantages of all methods for fractionation of humic substances, respectively humic systems or their singular fractions. One of the most promising methods for separation of humic systems seems to be the separation with molecular sieves. The fractionation by gel filtration is based on the fact that those molecules, of which the particle size is larger than the pores of the hydrated gel, go faster through the column by elution with a solvent than those molecules which are retained by diffusion inside the gel spheres. In the case of humic substances, the distribution occurs not only by the typical properties of molecular sieves but also by their sorption on the surface of the gel. An example for the reproducibility of separation is described (Fig. 7).

The gel filtration on Sephadex G 100 of humic substances from a podzol B-horizon with 0.04 N $Na_2\ B_4\ O_7$ leads to a curve which shows the distribution of possible particle weights or sizes. When one takes different fractions with always the same parts of volume during the elution procedure and separates again with the same techniques, then one gets distribution curves which correspond to the separated fractions. The summary curve of the single curves shows the same course as the original distribution curve of the total extract. This is also the case when one uses Gel 75 (below left).

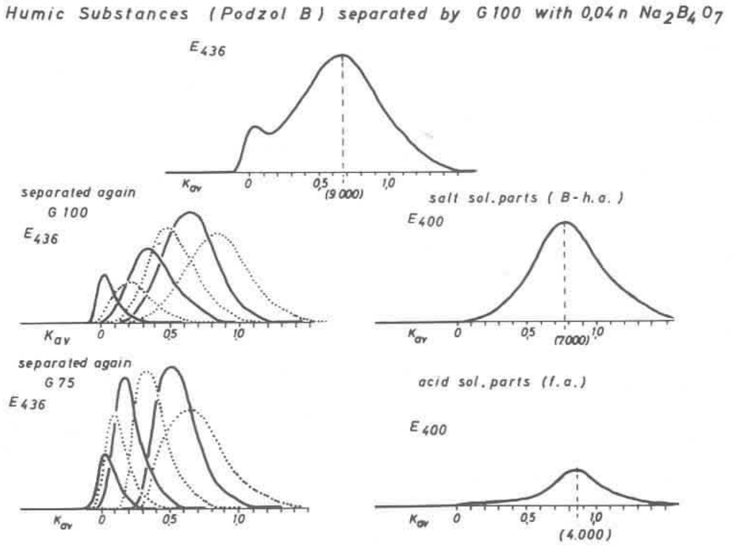

Fig. 7. Separation of humic substances from podzol B with 0,04 N $Na_2B_4O_7$ with Sephadex gel G 100 (Söchtig 1968)

When the different parts of the eluted solutions are made 1 N with Na_2SO_4, a part of the coloured substances, the "gray" humic acids fraction is precipitated with a found particle weight of about 9,000.

According to the definition, the salt soluble parts are the "brown" humic acids (B.-H. a.) and the fulvic acids (f. a.). The precipitates have been centrifuged and the light absorption of the supernatent has been measured. This part of the "brown" humic substances would have an average particle weight of 7,000.

When the different parts of the eluted solutions are acidified with sulpfhuric acid to pH 1, the acid soluble part, the fulvic acids shown on average particle weight of 4,000.

9. Some characteristics for the significance "humic acids" as a group of substances

In this section only some processes during the formation and some remarks about the properties of humic acids will be mentioned which are important to understand the difficulties for the elucidation of their constitution.

9.1. "Random" polymerisation

As has been mentioned above that several phenols from different origins participate in the formation of humic acids. Some of them can add other compounds nucleophilically, but others are not in the position but can be added. The addition of nitrogenous compounds occurs mainly by a secondary amino group.

The known polymers are formed by always repeating reactions of monomers by polymerisation and polycondensation. In the case of humic acids, a mechanism through free semiquinone radicals is supposed in which several monomers take part with different intermediate steps.

The hypothetical scheme of formation of melanin from tyrosine is mentioned to illustrate the "random" polymerisation of the free semiquinone radicals (Fig. 8).

The scheme illlustrates that the high molecular weight melanin is not formed by polymerisation of the end product of oxidation, the indole-5,6-quinone, but that all intermediates, which are formed during the oxidative polymerisation, are included in the reactions.

The nitrogen is also bound in a different manner as in the case of humic acids. It contributes not only by principal valences to the formation of the macromolecule but also by intermolecular valences which can lead to the formation of micelles.

One can consider that similar but also much more complicated reactions occur during the formation of humic acids because the reactivity of the phenols and the nitrogenous compounds is very different.

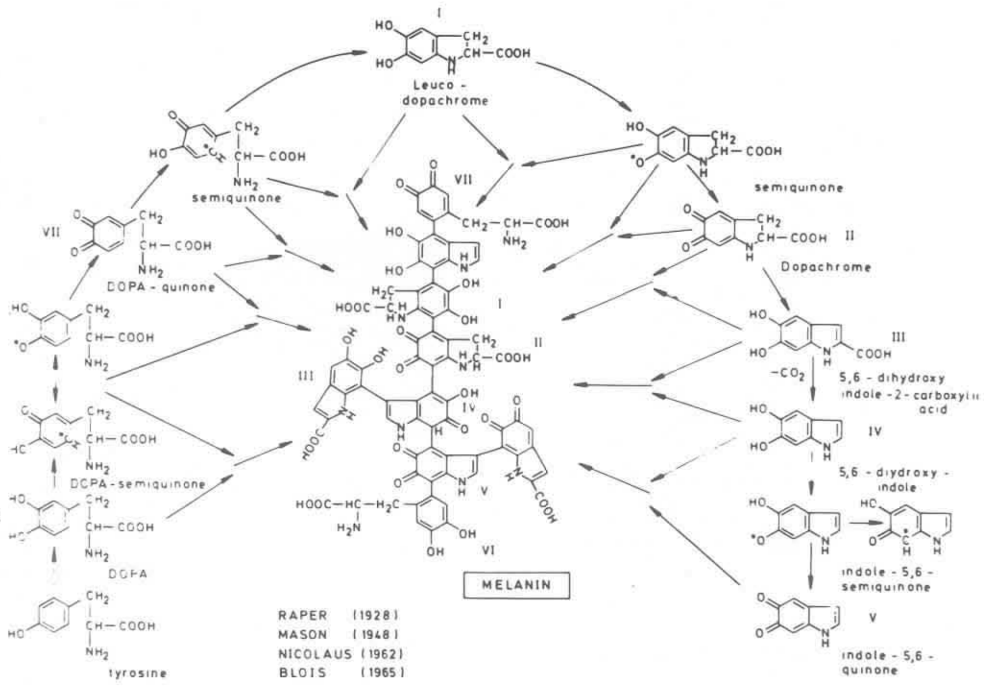

Fig. 8. Hypothetical scheme of melanin synthesis under participation of assumed free semiquinone radicals (Blois 1965)

9.2. Aggregation and dispersion

The scheme of "random" polymerisation has demonstrated that van der Waals forces occur in different groups. The strength of these forces are influenced by pH-value, concentrations of salts, solvents, temperature, the charge density, etc.

So an aggregation or dispersion of the humic acids depends for instance on the pH-value. Investigations with the electron microscope may illustrate this (Fig. 9) (Flaig and Beutelspacher, 1951; Beutelspacher, 1952).

Humic acids aggregate in acidic medium by alterations of their charge and hydration. Coorcervates are formed whereby several particles are surrounded with one water cover.

In alcaline medium dispersion occurs near single particles. In the present case, the particle diameter of humic acids from chernozem was calculated for about 80 to 100 Å. Similar values were also found by measurements with the ultracentrifuge.

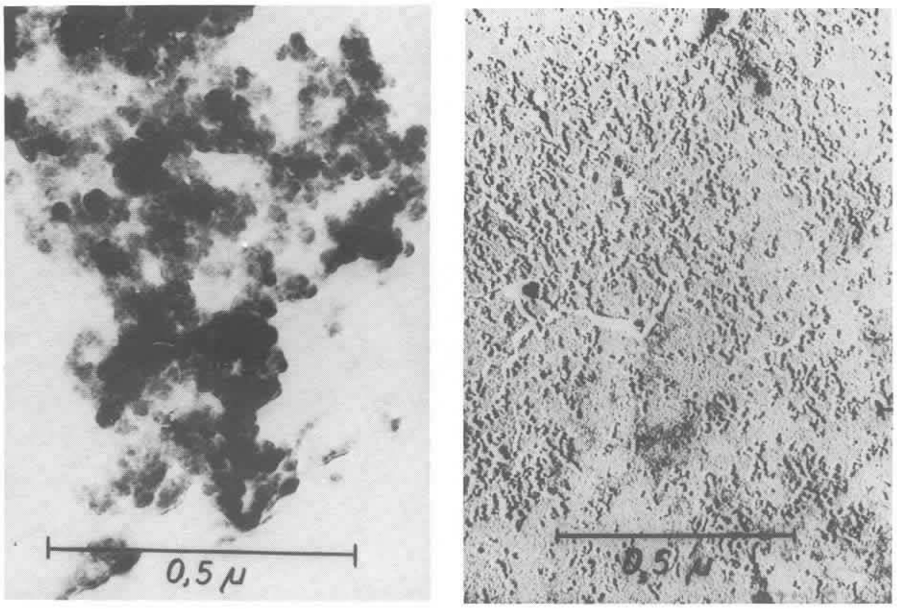

Fig. 9. Humic acids at pH = 3,5 (left) and pH = 8 (right)

Aggregation in dependence of pH could be established with viscosimetric measurements. At lower ph-values than 3,5, a sharp decrease of viscosity occurs. This may be explained by formation of micelles. The process of aggregation is reversible (Flaig and Beutelspacher, 1954). The viscosity decreases after diminution of hydrogen ion concentration.

The charge of the particles, which depends on pH-value, plays a role for investigations with the ultracentrifuge. The primary charge effect decreases in 1 % sodium chloride solution similar to the case of globular porteins (Flaig, and Beuteslpacher, 1968). The sedimentation constants are nearly doubled in comparison with aqueous suspensions, and the diffusion constants are reduced to a tenth. The molecular weights are between 60,000 and 77, 000 of the investigated fractions of humic acids at pH-values between 4,5, and 6.

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Discussion

Given: Professor Flaig has mentioned the reductive degradation of humic acids to a range of phenolic substances. I thought it might be of interest to mention that Dr. Jan Bimer in our laboratorys has applied this method to humic acids from peat and lignites, and also to humic acids produced by the oxidation of bituminous coals with performic acid. Attempts were made to analyse the phenols as TMS ethers by GLC. Although identifications were incomplete, it appears that a similar pattern of phenol structures persists even into bituminous coals, which implies a remarkable survival as chemical fossils of some of the structures and processes described by Professor Flaig.

Naucke: Herr Prof. Flaig, Sie haben einen umfassenden Abriß der modernen Humus-Chemie gegeben mit den Schwerpunkten

1. Vorstellungen über die Bildung von Huminstoffsystemen
2. Vorschläge für die Klassifizierung von Huminstoffen.

Ich möchte Sie zu Punkt 2 fragen, welche Huminstoff-Systeme Sie in den Kreisen Ihrer Untersuchungen bereits einbezogen haben?

Flaig: Wir untersuchen Huminstoffsysteme in verschiedenen Böden und Torfen. Es laufen zur Zeit auch einige Untersuchungen über die Veränderungen von Huminstoffsystemen während der Vegetation.

Teichmüller, M: Soviel ich weiß sind Grauhuminsäuren nur in Böden, aber nicht in Torfen und Braunkohlen festgestellt worden, Stimmt das, und wenn ja, welche Erklärung gibt es dafür?

Flaig: Die Huminsäuren aus Böden haben meistens einen höheren Stickstoffgehalt als die in Torfen und in Braunkohlen. Zu Ihrer Information darf ich Ihnen mitteilen, daß von Scharpenseel und Krause[1]) Grauhuminsäuren aus Braunkohlen beschrieben worden sind. Von diesen ist auch die Zusammensetzung der Aminosäuren bestimmt worden.

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[1]) Aminosäurenuntersuchungen an verschiedenen organischen Sedimenten, besonders Grau- und Braunhuminsäurefraktion verschiedener Bodentypen (einschließlich C^{14}-markierter Huminsäuren) – Z. Pflanzenernähr., Düngung, Bodenkunde.